## Title

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## Journal

The Journal of cell biology, 107(3)

## ISSN

0021-9525

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## Publication Date

1988-09-01

## DOI

10.1083/jcb.107.3.1207

Peer reviewed

# Expression of Apolipoprotein E by Cultured Vascular Smooth Muscle Cells Is Controlled by Growth State

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*Abstract.* Rat vascular smooth muscle cells (SMC) in culture synthesize and secrete a ~38,000-$M_r$ protein doublet or triplet that, as previously described (Majack and Bornstein. 1984. *J. Cell Biol.* 99:1688–1695), rapidly and reversibly accumulates in the SMC culture medium upon addition of heparin. In the present study, we show that this ~38,000-$M_r$ heparin-regulated protein is electrophoretically and immunologically identical to apolipoprotein E (apo-E), a major plasma apolipoprotein involved in cholesterol transport. In addition, we show that expression of apo-E by cultured SMC varies according to growth state: while proliferating SMC produced little apo-E and expressed low levels of apo-E mRNA, quiescent SMC produced significantly more apo-E (relative to other proteins) and expressed markedly increased levels of apo-E mRNA. Northern analysis of RNA extracted from aortic tissue revealed that fully differentiated, quiescent SMC contain significant quantities of apo-E mRNA. These data establish aortic SMC as a vascular source for apo-E and suggest new functional roles for this apolipoprotein, possibly unrelated to traditional concepts of lipid metabolism.

ATHEROSCLEROSIS is a complex disorder characterized by the abnormal proliferation of vascular smooth muscle cells (SMC)[1] and an altered lipid metabolism. Smooth muscle proliferation in the vessel wall appears to be controlled by a balanced interplay of endogenous and/or platelet-derived mitogens (38), trophic or accessory factors (such as lipoproteins; 12, 15, 19), and growth inhibitors (1, 5, 6, 8, 23). Among the known growth inhibitors are heparin-like glycosaminoglycans, endogenous components of the vessel wall (32) that act as potent regulators of SMC growth (5, 6, 7) and migration (26). Heparin-like molecules appear to regulate SMC growth in late $G_1$ (31) via a mechanism involving thrombospondin (27–30), an extracellular matrix glycoprotein whose synthesis is regulated by platelet-derived growth factor (27, 30) but whose deposition into the matrix is inhibited by heparin (27). Investigations of the effects of heparin on SMC function have revealed a variety of phenotypic alterations in heparin-treated SMC (24, 25, 27). In particular, heparin-treated SMC accumulate markedly increased amounts of a ~38,000-$M_r$ protein doublet in the culture supernatant (9, 24). The characteristics of regulation of this protein by heparin have been fully described (24).

In this study, we show that the ~38,000-$M_r$ heparin-regulated protein is identical to apolipoprotein E (apo-E), a major plasma apolipoprotein that plays a central role in cholesterol homeostasis and serves as a ligand for the apo-B, E (LDL) receptor (20, 21). Using electrophoretic, immunologic, and Northern hybridization techniques, we establish that aortic SMC synthesize and secrete apo-E and may therefore serve as an important extrahepatic source for this apolipoprotein. In addition, we demonstrate that apo-E production varies according to the proliferative state of SMC in culture. These data suggest an important link between SMC proliferation and vessel wall lipid metabolism, both key elements of the atherogenic process.

## Materials and Methods

### Cell Culture

Rat aortic SMC were grown from explants as described (27) and were subcultured in Waymouth's medium supplemented with 10% FCS (Gibco Laboratories, Grand Island, NY). Cells were used in the third to sixth passage. Unless otherwise indicated, the following culture conditions were employed. "Proliferative" SMC were prepared by plating cells at a density of 1–5 × $10^4$ cells/cm$^2$ and allowing them to proliferate for 24–48 h in 10% FCS. Growth factor-deficient (quiescent) SMC were prepared by maintaining similar cultures in 0.5% FCS for 72 h, or in 10% plasma-derived serum (PDS; HyClone Laboratories, Logan, UT) for 96 h (27, 28). "Overconflu-

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1. *Abbreviations used in this paper*: apo-E, apolipoprotein E; PDS, plasma-derived serum; SMC, smooth muscle cells.

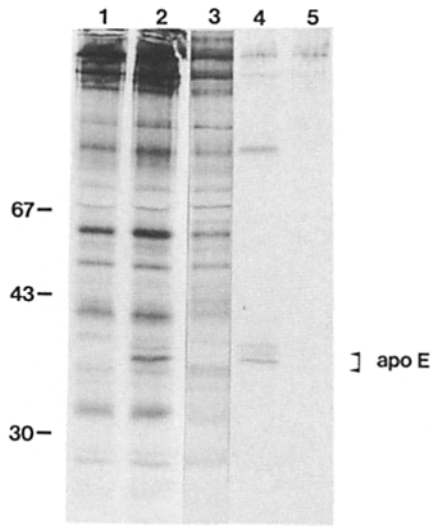


*Figure 1.* Secretory profiles of control and heparin-treated SMC and immunoisolation of apo-E from SMC culture medium. Proliferative rat aortic SMC were metabolically labeled with [$^{35}$S]methionine for 2 h (lanes *1* and *2*) or 16 h (lanes *3–5*). Heparin (100 μg/ml) was added to the labeling medium at the time of metabolic labeling (lane *2* only). Culture medium was collected and either processed directly for SDS-PAGE (lanes *1–2*) or treated with antiserum against rat plasma apo-E. Antigen–antibody complexes were precipitated with protein A–Sepharose as described. Radiolabeled and immunoisolated proteins were resolved on SDS-PAGE and visualized by autoradiography. Shown are secretory profiles of proliferative SMC labeled in the absence (lane *1*) or presence (lane *2*) of heparin. Note the selective increase in a ~38,000-$M_r$ protein doublet (see reference 26 for complete details). (Lanes *3–5*) Immunoprecipitation of SMC culture medium proteins with anti-apo-E antiserum. Lane *3*, starting material (profile of secreted proteins before antibody treatment). Lane *4*, secreted proteins immunoisolated with apo-E antiserum. (Note the specific precipitation of a protein triplet with ~37,000–39,000-$M_r$ that exactly comigrates with the heparin-inducible protein [lane *2*].) Lane *5*, secreted proteins precipitated by protein A–Sepharose alone (no antibody). A higher molecular mass protein (see in lane *4*) was occasionally observed to be precipitated with one of our antibodies; its identity is not known. The positions of migration of molecular mass standards ($\times 10^{-3}$) are shown to the left.

ent, nonproliferative" cultures were obtained by allowing the cells to reach overconfluent (>2 × $10^5$ cells/cm$^2$) densities in 10% FCS; growth curves revealed these cultures to be nonproliferative.

## Analytical Methods

Metabolic labeling of cellular and secreted proteins was performed as described (24, 25, 27) by culturing cells for 2–16 h in serum-free medium lacking methionine in the presence of 50 μCi/ml [$^{35}$S]methionine (New England Nuclear, Boston, MA). Labeled culture medium was harvested into protease inhibitors at 4°C to yield a final concentration of 25 mM EDTA, 45 μg/ml pepstatin, 1 mM *N*-ethylmaleimide, and 0.9 mM phenylmethylsulfonyl fluoride (PMSF). Proteins were precipitated in 10% TCA, dissolved in SDS-PAGE sample buffer (18), and resolved on 8 or 10% polyacrylamide gels. All samples were run in the presence of 50 mM dithiothreitol (DTT). Gels were fixed, dried, and exposed to film at −70°C. Radioactivity in autoradiographic bands was quantitated by optical density scanning using an Ephortec scanning densitometer (Joyce, Loebl and Co. Ltd., Malden, MA). Underexposed films were analyzed to insure densitometric readings in a linear range.

Immunoprecipitation from SMC culture medium was achieved by harvesting radiolabeled conditioned medium into PBS containing 0.1% SDS and 0.5% Triton X-100. Polyclonal antibodies against rat plasma apo-E or macrophage-derived apo-E, followed by protein A–Sepharose were used to specifically immunoisolate apo-E from the medium as described (16, 27). These antisera reacted only with apo-E when checked by immunoblotting against rat plasma apolipoproteins. Immunoisolated proteins were resolved by one-dimensional SDS-PAGE as described above, or by two-dimensional PAGE as described by O'Farrell (34) and modified by Skene and Shooter (40). Tube gels used were 1.5-mm-diam, containing 4% pH 3.5–10.0 and 2% pH 4–6 ampholytes (34).

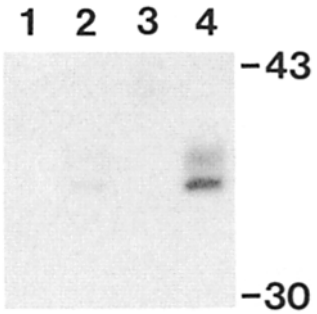


*Figure 2.* Effect of heparin on accumulation of the ~38,000-$M_r$ protein. Proliferative rat aortic SMC were metabolically labeled with [$^{35}$S]methionine for 2 h in the absence (lanes *1* and *2*) or presence (lanes *3* and *4*) of 100 μg of heparin/ml. Culture medium proteins were incubated with rabbit nonimmune serum (lanes *1* and *3*) or with rabbit anti-rat plasma apo-E antiserum (lanes *2* and *4*). Immunoreactive proteins were precipitated with protein A–Sepharose and resolved on SDS-PAGE with subsequent autoradiography. Note that heparin treatment enhances extracellular accumulation of the ~38,000-$M_r$ protein recognized by the apo-E antibody. The positions of migration of molecular mass standards ($\times 10^{-3}$) are shown to the right.

Total RNA was prepared from SMC by the SDS–proteinase K method (39). RNA was prepared from 150-mm-diam plates of SMC grown and treated as described, or from SMC enzymatically digested from the aortas of male Sprague-Dawley rats. The RNA samples (10 μg per lane) were glyoxylated before electrophoresis through an agarose gel formed in 10 mM phosphate, pH 6.8, then were electrophoretically transferred to Genescreen Plus hybridization membranes (DuPont Co., Wilmington, DE; New England Nuclear). The filters were UV cross-linked and prehybridized in 0.5 M sodium phosphate (pH 7.2) containing 1 mM EDTA, 1% BSA, and 7% SDS (7). DNA probes were then added (~5 × $10^7$ dpm/ml) and allowed to hybridize at 65°C for 12–20 h. Filters were washed in two changes of 0.1× SSC/1% SDS at 65°C, then were exposed to XAR-5 film (Eastman Kodak Co., Rochester, NY) with Cronex intensifying screens (DuPont Co.) at −70°C.

DNA probes were labeled with [$^{32}$P]dCTP to a specific activity of 2–5 × $10^5$ dpm/ng by random priming, using an oligo-labeling kit (Pharmacia Fine Chemicals, Piscataway, NJ) and following the instructions supplied by the manufacturer. The DNA probes used were 300-bp Pst I restriction fragments of pGR124, a cDNA encoding rat apolipoprotein E (33), and a 1.4-kb BamHI fragment of pPAI-1, a cDNA encoding human type I plasminogen activator inhibitor (a gift of Drs. L. Erickson and K. Marroti). The plasminogen activator inhibitor probe recognized a single 2.3-kb transcript that is constitutively expressed by SMC in culture (30), and was used as a control for RNA loading and transfer.

## Results

Rat aortic smooth muscle cells in culture synthesize and secrete a ~38,000-$M_r$ protein doublet or triplet that accumulates in the culture supernatant in response to heparin treatment (Fig. 1). The selective increase in medium levels of this protein occurred without a concomitant change in total protein synthesis (24, 25), and was independent of the effects of heparin on SMC growth (24). SMC also synthesize and secrete a ~38,000-$M_r$ protein doublet or triplet identified immunologically as apo-E (Fig. 1).

We have investigated the relationship between the heparin-regulated ~38,000-$M_r$ protein and apo-E, using immunologic and electrophoretic criteria. Confluent, nonproliferating SMC were metabolically labeled for 2 h with [$^{35}$S]methionine in the presence or absence of 100 μg of heparin/ml.

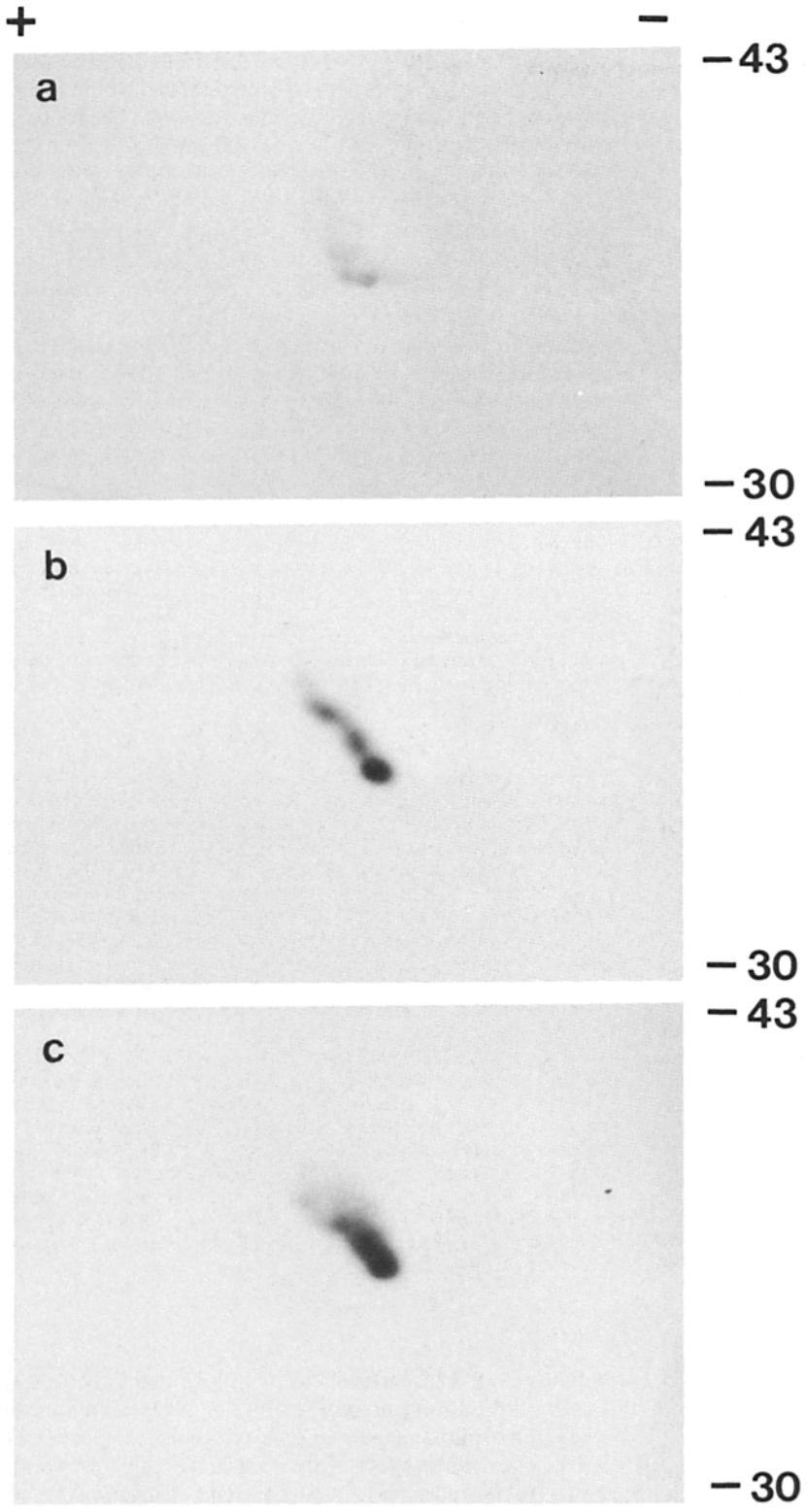


*Figure 3.* Two-dimensional PAGE analysis of plasma apo-E, aortic SMC-derived apo-E, and macrophage-derived apo-E. Metabolically labeled SMC-derived apo-E was immunoisolated from SMC culture medium as described (see legend to Fig. 2). Before electrophoresis, unlabeled rat plasma apo-E was added to the sample, and the resulting gel was stained with Coomassie Blue and photographed before autoradiography. Metabolically labeled macrophage-derived apo-E was prepared from supernatants of a glial cell culture containing oligodendrocytes, astrocytes, and macrophages. Samples were subjected to two-dimensional PAGE as described in Materials and Methods. (*a*) Coomassie Blue–stained plasma apo-E. (*b*) Autoradiograph of apo-E derived from cultured rat aortic SMC. (*c*) Autoradiograph of apo-E derived from cultured macrophages. The slight difference in the electrophoretic pattern of plasma apo-E (due to increased amounts of an isoform in the −1 position with a molecular mass similar to nonglycosylated apo-E) is commonly observed with isolated apo-E and is most likely related to deamidation during storage (44). The positions of migration of molecular mass standards ($\times 10^{-3}$) are shown to the right.

Equal amounts of radioactively labeled culture medium were treated sequentially with anti–apo-E antiserum and protein A–Sepharose and the precipitated proteins were resolved by SDS-PAGE. As shown in Fig. 2, the culture medium from heparin-treated SMC contained large quantities of the ~38,000-$M_r$ protein precipitated by the apo-E antiserum, whereas very little immunoprecipitable apo-E was identified in the culture supernatants of SMC labeled in the absence of heparin. The identity of the ~38,000-$M_r$ protein as apo-E was confirmed in these studies using two different apo-E antisera prepared against plasma apo-E, as well as with an antibody prepared against macrophage apo-E.

The immunoisolated ~38,000-$M_r$ protein from the culture medium of heparin-treated SMC was compared to plas-

ma apo-E and macrophage-secreted apo-E by two-dimensional PAGE. Plasma apo-E is composed of a major band that represents the nonglycosylated form of the protein and variably sialylated forms that are more acidic and migrate with an increasing apparent molecular mass (producing the stairstep effect seen in Fig. 3 *a*; 37, 44). As shown in Fig. 3 *b*, the SMC-derived protein separated into three components with a pattern similar to that of plasma apo-E. In addition, the pattern seen with apo-E derived from macrophages was very similar (Fig. 3 *c*). An increased degree of sialylation of macrophage apo-E, as compared to plasma apo-E, has been previously reported (2, 43) and correlates with the increased amounts of the apparently higher molecular mass, acidic forms immunoprecipitated from the SMC and macrophage culture media. No radiolabeled proteins corresponding to apo-E could be precipitated from SMC culture medium treated with nonimmune serum (data not shown). On the basis of these data, we concluded that the SMC-derived, heparin-inducible ~38,000-$M_r$ protein was apo-E.

As shown in our previous studies (24) and in Table I, addition of heparin to SMC cultures results in a rapid accumulation of apo-E in the SMC culture medium. We have used Northern blot analysis to estimate the amount of apo-E mRNA in control and heparin-treated SMC, to determine if the increase in extracellular apo-E content was accompanied by a concomitant increase in apo-E transcripts. RNA was prepared from overconfluent, nonproliferative cells in the absence or presence of heparin (100 μg/ml for 72 h). As in our previous studies (24), these nonproliferative conditions were selected in an attempt to dissociate the direct effects of heparin from other effects secondary to SMC growth inhibition. As shown in Fig. 4, heparin treatment did not result in an increase in apo-E mRNA levels under nonproliferative conditions, despite a marked accumulation of secreted apo-E in the heparin-treated cultures (24). These data suggest that heparin exerts a posttranscriptional or postsecretional effect on apo-E accumulation in the medium. However, when RNA was prepared from sparse proliferating cells and from sparse cells growth-inhibited by heparin, a marked increase in apo-E message levels was observed in the heparin-treated cultures (Fig. 4). Thus apo-E mRNA levels appear to increase as a function of growth inhibition rather than heparin treatment. On the basis of these and previous (24) data, we conclude that the effects of heparin on apo-E production may occur at two levels: directly, via a posttranscriptional or postsecretional effect, and indirectly, via the establishment of a quiescent state.

We next examined the role of quiescence in determining apo-E expression by SMC, using metabolic labeling techniques. SMC were made quiescent by maintaining the cultures in 0.5% FCS for 72 h or in 10% PDS for 96 h (27, 28). The nuclear labeling indices (over a 30-h period) of SMC maintained under these conditions was found to be <10% (29). As shown in Fig. 5, the apo-E content of the media from quiescent SMC was markedly higher than that from proliferative cells at the same cell density. Addition of heparin further increased the accumulation of newly synthesized apo-E in the quiescent cells (Fig. 5). Scanning densitometry was used to quantitate the relative levels of apo-E secreted by SMC under various culture conditions (Table I). Cells rendered quiescent in 0.5% FCS or 10% PDS produced an average of four- to fivefold more apo-E (as a percent of total secreted protein) than did proliferative cells. The addition of heparin to proliferating SMC resulted in a 10-fold increase of accumulated apo-E in the culture supernatant. Note that this increase is very rapid and occurs within 2 h (Table I and reference 24), well before the antiproliferative effects of heparin become apparent. Heparin treatment of quiescent SMC elicited a similar increase in extracellular apo-E levels (Table I).

To determine if the enhanced production of apo-E by quiescent SMC correlated with an increase in apo-E mRNA levels, we compared RNA prepared from proliferative SMC with RNA prepared from SMC growth arrested in 0.5%

*Table I. Accumulation of Secreted Apo-E by Vascular SMC as a Function of Quiescence or Heparin Treatment*

| | Apo-E (in relative levels)* |
|---|---|
| Control (10% FCS) | 1.8 ± 0.6 |
| + heparin (100 μg/ml; 2 h) | 20.3 ± 3.2 |
| Quiescent (10% PDS; 96 h) | 6.8 ± 2.6 |
| Quiescent (0.5% FCS; 72 h) | 8.6 ± 1.5 |
| Quiescent (0.5% FCS) + heparin | 25.5 ± 3.0 |

* Expressed as a percent of total secreted radioactivity as determined by scanning densitometry of SDS-PAGE autoradiographs.

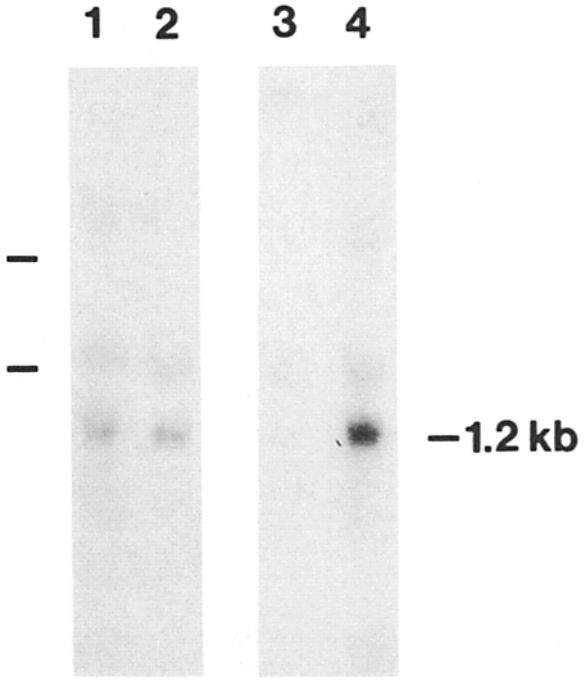


*Figure 4.* Northern analysis of RNA prepared from control and heparin-treated SMC under proliferative and nonproliferative (overconfluent) conditions. Total RNA was prepared from overconfluent, nonproliferating SMC cultures (lane *1*), overconfluent, nonproliferating SMC treated for 72 h with 100 μg of heparin/ml (lane *2*), sparse, proliferating SMC (lane *3*), and sparse proliferating SMC after growth arrest with heparin for 72 h (lane *4*). These RNAs (10 μg/lane) were subjected to Northern analysis with $^{32}$P-labeled rat apo-E cDNA probe. The apo-E cDNA recognizes a single SMC mRNA species at 1.2 kb. Note that heparin has no direct effect on apo-E message levels in nonproliferative cultures under conditions in which extracellular apo-E levels are greatly induced (24). Apo-E mRNA content is markedly induced, however, in sparse SMC growth-arrested by heparin. Bars (*left*) indicate the positions of migration of 28S and 18S ribosomes.

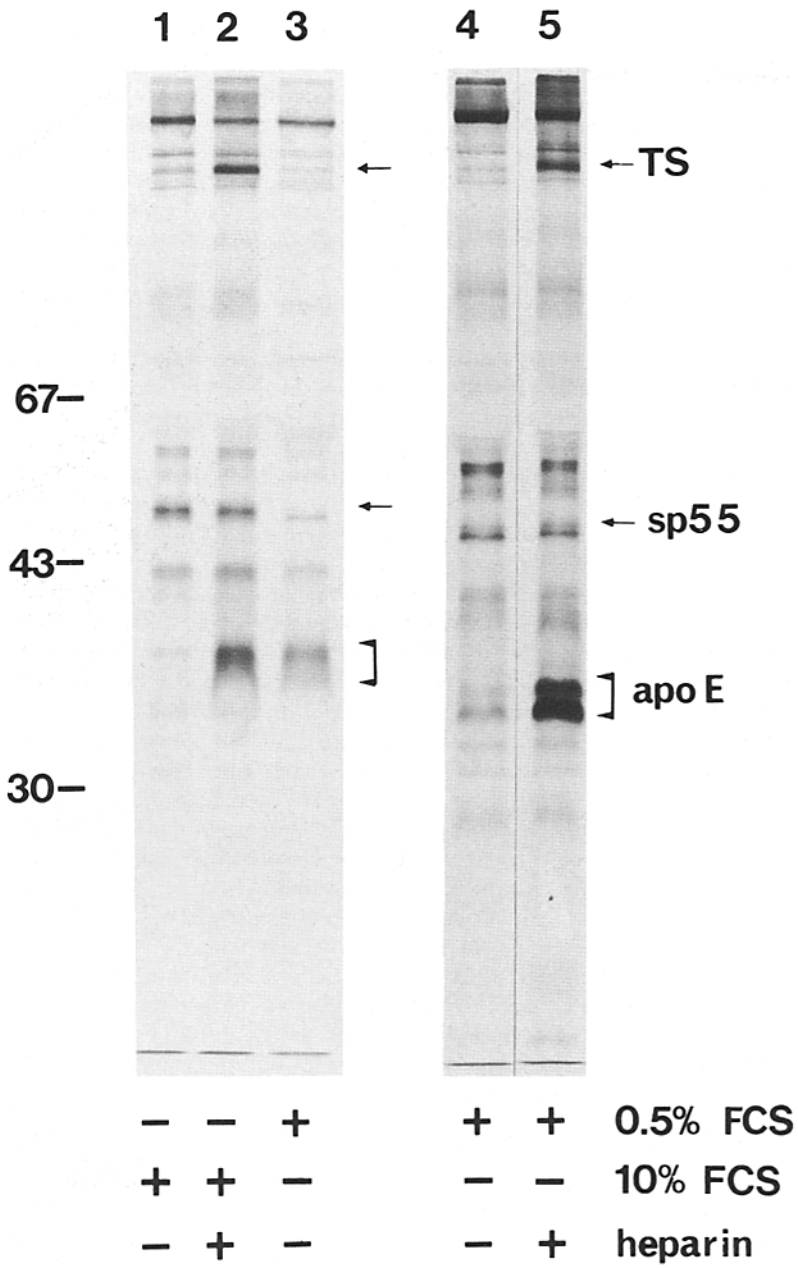


*Figure 5.* Expression of apo-E by SMC as a function of growth state and heparin treatment. Secreted SMC proteins were metabolically labeled as described, resolved on SDS-PAGE, and visualized by autoradiography. Shown are secretory phenotypes of SMC proliferating in 10% FCS (lane *1*), proliferating SMC treated with heparin for 2 h (lane *2*), and SMC rendered quiescent in 0.5% FCS for 72 h (lane *3*). (Lanes *4* and *5*) Secretory phenotypes of 0.5% FCS-arrested SMC labeled for 2 h in the absence (lane *4*) or presence (lane *5*) of heparin. Enhanced production of thrombospondin (*TS*) and a secreted ~55,000-$M_r$ protein (*sp 55*) serve as markers for growth stimulation by FCS (lanes *1* and *2*). Medium TS levels are also increased in heparin-treated cells (lanes *2* and *5*; see reference *27* for details). Bracket indicates the position of migration of apo-E in the two examples shown here. Note enhanced expression of apo-E in heparin-treated and growth-arrested cells. The positions of migration of molecular mass standards ($\times 10^{-3}$) are shown to the left.

FCS. As shown in Fig. 6, quiescent SMC in culture contain significantly elevated amounts of apo-E mRNA as compared to proliferating controls. These data are consistent with the data derived from the metabolic labeling experiments described above, which demonstrated markedly increased production of apo-E by quiescent cells. The data presented in Fig. 6 are derived from cells growth arrested in 0.5% FCS for 72 h; a similar increase in apo-E mRNA was also observed in cells made quiescent in 10% PDS (not shown). SMC isolated directly from normal aortic tissue also contained substantial levels of apo-E message (Fig. 6), suggesting that apo-E production is a normal differentiated function of quiescent SMC in vivo.

## *Discussion*

Apo-E is the protein moiety of several classes of plasma lipoproteins and serves as the ligand responsible for mediating their binding to the apo B,E (LDL) receptor (for reviews see references 20 and 21). Apo-E is synthesized by a variety of cell types, including macrophages and astrocytes (3, 4, 10, 11, 20), and may function in cholesterol transport among various cells in the body, especially during tissue growth or repair (14, 17). For example, high levels of macrophage-derived apo-E have recently been shown to accumulate in injured peripheral nerves during periods of degeneration and regeneration (16, 42; Boyles, J. K., D. Y. Hui, K. H. Weisgraber, R. E. Pitas, and R. W. Mahley, unpublished data); this apo-E is believed to participate in the redistribution of cholesterol derived from degenerating myelin (Boyles, J. K., D. Y. Hui, K. H. Weisgraber, R. E. Pitas, and R. W. Mahley, unpublished data). Other (nonlipid transport) roles for apo-E in tissue injury and repair have not, however, been excluded. In this study we demonstrate that rat aortic SMC express

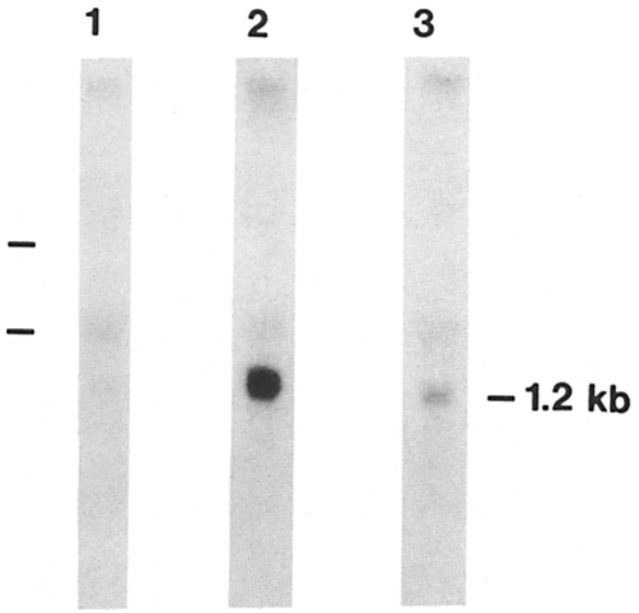


*Figure 6.* Northern analysis of RNA prepared from proliferating and quiescent cultures, and from SMC isolated from an in vivo source. Total RNA was prepared from sparsely plated proliferative SMC (lane *1*), sparse SMC growth-arrested by serum deprivation (72 h in 0.5% FCS (lane *2*), and SMC enzymatically released from the abdominal aortas of 4-mo-old male rats (lane *3*). These RNAs (10 μg/lane) were subjected to Northern analysis with a $^{32}$P-labeled rat apo-E cDNA probe. Cells growth arrested by culture in low (0.5%) serum for 72 h (lane 2) exhibit markedly increased levels of apo-E mRNA. SMC isolated from aortic tissue in vivo also express a significant amount of apo-E message. Bars (*left*) indicate the positions of migration of 28S and 18S ribosomes.

apo-E mRNA and, in culture, synthesize and secrete a protein with electrophoretic and immunologic identity to plasma and macrophage-derived apo-E. In addition, we establish identity of apo-E with a previously described ~38,000-$M_r$ protein that accumulates in the SMC culture medium in response to heparin (25), and show that apo-E expression is enhanced during SMC quiescence.

A recent paper by Driscoll and Getz (10) demonstrated that SMC cultured from monkey aorta synthesize and secrete a protein recognized by an antibody against plasma apo-E. Using rat aortic SMC, we have rigorously proven (using three apo-E antisera, two-dimensional PAGE analysis, and Northern hybridization techniques) that SMC in culture produce apo-E. In addition, we show that aortic SMC in vivo contain significant amounts of apo-E mRNA. Our data clearly establish SMC as an additional extrahepatic source for apo-E. Production of this apolipoprotein directly by vascular tissues may be of particular relevance to the atherogenic process, which is characterized by cellular proliferation (requiring de novo membrane synthesis) and an abnormal lipid metabolism.

Heparin-like molecules are endogenous components of the vessel wall (32) that probably exist in the extracellular matrix in the form of heparan sulfate proteoglycans. Current theories emphasize the "protective" role that these molecules may serve in prohibiting or controlling SMC proliferation in vivo after vascular injury or trauma (6). In culture, SMC exposed to heparin accumulate large amounts of the ~38,000-$M_r$ protein (9, 24) identified herein as apo-E. Heparin is known to bind apo-E (22, 42) and can prevent the interaction of apo-E–containing lipoproteins with the apo-B,E (LDL) receptor (13). It is likely that heparin contributes to the accumulation of apo-E in SMC cultures by a postsecretional mechanism, either by inhibiting the receptor-mediated uptake of secreted apo-E or by binding to the apo-E and preventing its extracellular degradation. This hypothesis is supported by the very rapid kinetics of induction after addition of heparin to the SMC culture medium (24), the immediate reversal of the effect upon removal of heparin (24), and the absence of detectable change in apo-E mRNA after prolonged heparin treatment of confluent cells (Fig. 4). If a similar phenomenon occurs in vivo, the presence of endogenous extracellular heparans may serve an important regulatory function in vascular lipid metabolism.

In addition to these probable postsecretional effects, heparin treatment appears to exert an indirect effect on the expression of apo-E via the induction of cellular quiescence. We have found that sparse SMC growth arrested by heparin exhibit increased apo-E mRNA in the absence of a direct effect of heparin on apo-E message levels under nonproliferative (overconfluent) culture conditions. Expression of apo-E by cultured SMC therefore appears to be controlled, at least in part, by growth state. In support of this hypothesis, we have shown that SMC maintained in low (0.5%) or plasma-derived serum (which lacks platelet products) secrete increased amounts of apo-E. The increased production of apo-E was accompanied by a concomitant increase in apo-E mRNA levels, and correlated with the establishment of a quiescent state due to growth factor deprivation. These observations, together with our demonstration of significant levels of apo-E mRNA in aortic tissue in vivo, may relate to the findings of Owens et al. (35, 36), who suggest that growth arrest (by serum deprivation) promotes the cytodifferentiation of cultured SMC. Our data clearly establish that apo-E gene expression is a constitutive function of fully differentiated SMC, and that expression of this differentiated function is enhanced by quiescence in culture. More importantly, the data suggest that stimulation of SMC proliferation in vivo (e.g., after vascular injury) may lead to profound alterations in vessel wall lipid metabolism.

In summary, we have shown that aortic SMC synthesize and secrete apo-E, and that apo-E is the previously studied ~38,000-$M_r$ protein regulated by heparin in SMC cultures (9, 24). The mechanism underlying the increased accumulation of secreted apo-E in heparin-treated, nonproliferating (overconfluent) cultures was not examined but most probably occurs at a postsecretory level. We have also shown that cells maintained in low or plasma-derived serum secrete four- to fivefold more apo-E than do proliferating cells. The increased apo-E production occurred concomitant with growth arrest and was associated with a marked increase in apo-E mRNA. Heparin-mediated inhibition of SMC growth also resulted in increased apo-E message levels. Taken together, the available data suggest intriguing relationships among apo-E, endogenous vessel wall heparans, and SMC growth and/or differentiation. Further studies on the functional role of apo-E in SMC biology, on the functional consequences of interactions between heparin and apo-E, and on relationship(s) among SMC proliferation, apo-E gene expression, and vascular lipid metabolism are clearly indicated.

We thank J. L. Obreiter for excellent secretarial assistance; Dr. G. Melchior for a critical reading of the manuscript; Dr. L. Dory for a generous gift of antiserum against rat plasma apo-E; Drs. L. Erickson and K. Marotti for providing the PAI-1 cDNA probe; and Dr. J. W. Taylor for providing the cDNA probe for apo-E.

This work was supported in part by a grant from the Max-Planck-Society, Munich, Federal Republic of Germany to P. J. Gebicke-Haerter and by the Isabelle Niemala Fund to E. M. Shooter.

Received for publication 16 February 1988, and in revised form 2 May 1988.

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